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Site-Specific Insertion of IS*1301* and Distribution in *Neisseria meningitidis* Strains

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The insertion element IS*1301* has been shown to mediate capsule phase variation in *Neisseria meningitidis* serogroup B by reversible insertional inactivation of the *siaA* gene. We have determined the target site specificity of this element by cloning and sequencing the insertion sites of 12 identical IS*1301* copies found in *N. meningitidis* B1940. A target consensus core of 5′-AYTAG-3′ was identified, with the central TA being duplicated following insertion. Additional features around the target sites, including extended palindromic symmetry, stem-loop formation, and the high incidence of AT tracts, indicate that other factors, such as DNA secondary structure, are involved in target recognition. The left inverted repeat of an IS*1016*-like element acts as a hot spot for insertion, with one insertion element combination located upstream of the *frpC* gene. According to further sequence analysis, we were able to place IS*1301* in the IS*5* subgroup within the IS*4* family of elements. A survey of 135 *Neisseria* strains indicated the presence of IS*1301* in 27.9 to 33.3% of *N. meningitidis* serogroup B, C, and W135 strains and in 86.7% of serogroup Y strains. IS*1301* did not occur in serogroup A strains, in *Neisseria gonorrhoeae*, or in apathogenic *Neisseria* spp.

Mobile genetic elements are found in many different genetic entities in which they are involved in genetic rearrangement and genome plasticity. Transposition of insertion elements (IS) is known to cause a number of effects, including insertions, deletions, and cointegrate formation, resulting either in silent mutations, in knockout of gene expression (14), or in regulation of downstream-located genes (5, 14, 34). Transposition activity may thus create diversity in a bacterial population, with the result that a small fraction of the bacteria is preadapted to environmental changes (2, 21).

By use of an epithelial cell invasion model, the regulation of capsule phase variation in the gram-negative diplococcus *Neisseria meningitidis* serogroup B was recently demonstrated by site-specific insertion and precise excision of IS*1301* within the *siaA* gene (19) of the capsular polysaccharide biosynthesis pathway (9). This 842-bp element is flanked by 19-bp inverted repeats (IR) and contains two overlapping open reading frames (ORFs), possibly coding for proteins with 151 and 179 amino acid residues. IS*1301*-mediated loss of encapsulation results in both stronger adherence and increased entry of meningococci into epithelial cells (19, 42). Since precise excision of IS*1301* restores the formation of the capsule and thus enables meningococci to survive once they have entered the bloodstream, this reversible mechanism may be of importance for the understanding of invasive meningococcal disease.

Although a huge number of IS in bacteria have been identified, besides IS*1301*, only a few examples of reversible regulatory functions of gene expression have been described: IS*492* causes reversible inactivation of extracellular polysaccharide production in *Pseudomonas atlantica* (1, 2), IS-*PA-4*/*5* is involved in the reversible mucoid conversion of *Pseudomonas aeruginosa* (36), IS*1* insertion in *virF* of *Shigella flexneri* results in loss of virulence (26), and insertion of an IS*1*-like element causes inactivation of the *Citrobacter freundii* capsular antigen Vi in *Escherichia coli* (29). However, except for IS*1301* in *siaA* and IS*492* in the *eps* locus, the exact mechanisms of regulation remain unclear, either with the link between insertion site and gene expression missing (IS-*PA-4*/*5*) or with only indirect evidence for reversibility (IS*1* in *virF*) or no evidence in the natural host (Vi of *C. freundii*).

IS-mediated regulation of genes requires a certain degree of insertion specificity of the IS-encoded protein needed for transposition, called transposase. However, little is known about the mechanisms by which the transposition complex chooses the insertion site at the target DNA. Whereas most elements show a preference for defined regions in the genome that are not obviously related in sequence, the recognition of the insertion site of a few IS appears to be determined by a distinct target sequence (14). Interestingly, many insertions target a central 5′-TA-3′ dinucleotide within palindromes. For example, bacterial insertion sequence IS*630*, as well as *Tc1* from *Caenorhabditis elegans*, always transposes to 5′-TA-3′ dinucleotides, in the case of IS*630* preferentially within 5′-CTAG-3′ (27, 38, 39), and IS*711* from *Brucella ovis* transposes to the 5′-YTAR-3′ motif (18). While the flanking sequences did not influence the transposition of IS*630*, an S-shaped DNA bending centered around the target site was required for sufficient transposition of IS*231*A from *Bacillus thuringiensis* in addition to the sequence of the target consensus, 5′-GGG$(N)_5$CCC-3′ (17).

IS*1301* specifically recognizes one of 12 5′-TTAG-3′ tetranucleotides located in *siaA* (9, 19). In this report, we present additional sequence data for 12 other insertion sites of the element different from the *siaA* gene in *N. meningitidis* B1940. The consensus sequences 5′-ACTAG-3′ and 5′-ATTAG-3′ were determined to be target sites for IS*1301*, but additional characteristics of the surrounding sequence influence the target specificity.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *N. meningitidis* B1940 (U. Berger, Institut für Hygiene, University of Heidelberg, Heidelberg, Germany) was a disease isolate and was typed as B:NT:P1.3,6,15, lipopolysaccharide im-

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munotype L3,7,9. For analysis of IS*1301* distribution, 118 additional meningococcal strains, including serogroups A, B, C, Y, and W135, as well as three gonococcal strains and 14 strains of apathogenic *Neisseria* (see Table 1) were screened; 89 meningococcal strains collected from all over Germany between 1993 and 1994 and the apathogenic *Neisseria* strains were from the National Reference Center for *Neisseria* (at the Institut für Hygiene, University of Heidelberg). Other strains were kindly provided by M. Achtman (Max-Plank Institut für Molekulare Genetik, Berlin, Germany), D. A. Caugant (National Institute for Public Health, Oslo, Norway), A. Fox (Manchester Public Health Laboratory, Manchester, United Kingdom), and W. Zollinger (Walter Reed Army Institute of Research, Washington, D.C.) or were collected in our own institution. All *Neisseria* strains were grown on chocolate agar at 37°C under 5% CO_2. *E. coli* Sure and plasmid pBluescript SK+ were obtained from Stratagene (Heidelberg, Germany). *E. coli* was grown at 37°C on Luria-Bertani agar with the addition of 100 μg of ampicillin per ml, when appropriate.

Recombinant DNA techniques. Total DNA from *N. meningitidis* was isolated and purified as described previously (37). Minipreparation of recombinant plasmids was performed by the alkaline lysis method (33). DNA was digested with restriction enzymes purchased from Pharmacia (Freiburg, Germany) according to the instructions of the manufacturer. DNA fragments were separated by agarose gel electrophoresis (33) and were recovered from agarose gel slices with a Jetsorb gel purification kit (Genomed, Bad Oeynhausen, Germany) according to the instructions of the manufacturer. A genomic library of *N. meningitidis* was constructed by partial digestion of chromosomal DNA with *Sau*3A and subsequent ligation of 1- to 2-kb fragments into the *Bam*HI site of plasmid pBluescript SK+ or by complete digestion with *Hin*cII and subsequent ligation of 2- to 6-kb fragments into the *Hin*cII site of the vector followed by transformation into *E. coli* Sure. T4 ligase was purchased from Gibco-BRL (Gaithersburg, Md.).

Macrorestriction analysis. Preparation of chromosomal DNA and restriction endonuclease digestion were performed essentially as described previously (13). Briefly, bacteria were grown overnight, harvested with a cotton swab, resuspended in SE buffer (75 mM NaCl, 25 mM EDTA; pH 7.5), and enclosed in 1% (wt/vol) (final concentration) low-melting-temperature agarose blocks. The blocks were incubated with 0.5 mg of proteinase K per ml in ES buffer (0.5 M EDTA, 1% [wt/vol] *N*-lauroylsarcosine; pH 7.5) overnight at 50°C and washed at least three times in TE (10 mM Tris-HCl, 10 mM EDTA; pH 8.0). For restriction analysis, blocks were digested overnight at 37°C in the appropriate restriction buffer supplemented with 6.5 mM dithioerythriol, 135 μg of bovine serum albumin, and 10 U of the restriction enzyme in a total volume of 150 μl. One-dimensional pulsed-field gel electrophoresis was performed with a contour-clamped homogeneous electric field DR II apparatus (Bio-Rad Laboratories, Munich, Germany) with 1% agarose gels, using a linear ramp of 1 to 35 s for 21 h. Concatemers of phage λ*c*I857 *S*am7 DNA (Bio-Rad) served as molecular weight markers. DNA was nicked before being transferred to nylon membranes by UV illumination at 305 nm for 90 s.

Southern blot analysis and colony blot hybridizations. For Southern blot analysis, DNA was depurinated, denatured, and transferred to nylon membranes (Qiagen, Hildern, Germany) essentially as described previously (33). Colony blotting was performed following transfer to nylon membranes, alkaline lysis of bacteria (33), UV immobilization of DNA, and additional washing in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.5% sodium dodecyl sulfate (SDS)–1 mM EDTA to remove bacterial debris. DNA probes were labeled with random primers by using a digoxigenin system obtained from Boehringer (Mannheim, Germany) according to the instructions of the supplier. Hybridization was performed at 42°C in high-SDS buffer, containing 7% SDS, 50% formamide, 5× SSC, 50 mM sodium phosphate (pH 7.0), 0.1% *N*-lauroylsarcosine, and 2% blocking reagent (Boehringer). Hybridized probes were detected by using a chemiluminescence detection kit from Boehringer according to the instructions of the manufacturer.

PCR. Goldstar *Taq* polymerase was purchased from Eurogentec (Seraing, Belgium) and used under buffer conditions recommended by the manufacturer, including 20 μM each deoxynucleoside triphosphate, 1.25 U of polymerase, and 20 pmol of each primer in a 50-μl reaction volume. Primers were derived from the IR sequences flanking IS*1301*, thus amplifying the whole element of 842 bp: SH52 (5′-GGGCGTGTCCCTAATTTGA-3′) and SH53 (5′-GGGCGTGTCCT CAATTTAAC-3′). PCR was performed in a Thermocycler (Landgraf, Hannover, Germany) using the following parameters: initial denaturation for 3 min at 94°C followed by 30 cycles of denaturation for 60 s at 94°C, annealing for 60 s at 53°C, and elongation for 60 s at 72°C.

DNA sequencing. DNA sequencing was performed by the dideoxy termination method using T7 DNA polymerase (Pharmacia, Freiburg, Germany). Sequences flanking the insertion sites of IS*1301* were determined by using outward-directed primers located inside IS*1301*, RH1 (5′-TCTTCCATTGATGACAGCCG-3′, positions 143 to 162; reverse) and RH2 (5′-ATTACGCAACTTAGTCGAGA-3′, positions 695 to 714). All oligonucleotide primers were purchased from Pharmacia. Sequence data were analyzed with PC/GENE software (IntelliGenetics, Mountain View, Calif.).

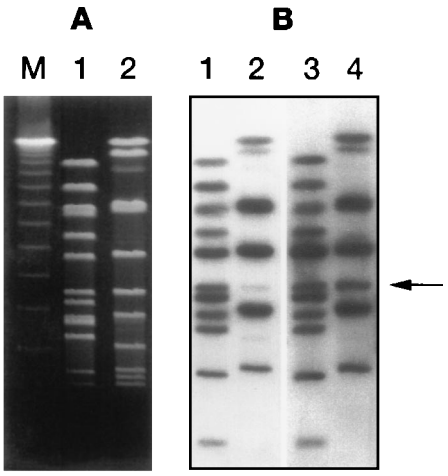

FIG. 1. Macrorestriction fragment analysis of *N. meningitidis* B1940. (A) Restriction fragment pattern of B1940 chromosomal DNA digested with *Nhe*I (lane 1) and *Sfi*I (lane 2), separated by pulsed-field gel electrophoresis, and stained with ethidium bromide; the encapsulated wild-type strain and the unencapsulated IS*1301*::*siaA* variant are indistinguishable. Concatamers of phage λ*c*I857 *S*am7 were used as size markers (lane M); the size of the monomer is 48.5 kbp. (B) Lanes 1 and 3, *Nhe*I digest; lanes 2 and 4, *Sfi*I digest. DNA fragments of the wild-type strain (lanes 1 and 2) and the nonencapsulated variant (lanes 3 and 4) were transferred to a nylon membrane and hybridized to an IS*1301*-specific probe. In both the wild type and the unencapsulated variant, the IS is present on 11 of 14 *Nhe*I fragments, indicating a random distribution in the genome without obvious clusters. An additional band is observed in the variant following digestion with *Sfi*I (lane 4, arrow); the localization in the genomic map matches the capsule gene cluster (13).

RESULTS

IS*1301* copies are not clustered in *N. meningitidis* B1940. Southern hybridizations indicated the presence of at least 12 copies of IS*1301* in the genome of *N. meningitidis* B1940 (19). By Southern blotting and hybridization of a macrorestriction assay of the *N. meningitidis* B1940 genome with an IS*1301*-specific probe, we were able to locate the different copies of the element, using a recently published genome map of this strain (13). The results are shown in Fig. 1, indicating a scattered distribution over the whole genome. As expected, the additional band observed in the nonencapsulated IS*1301*::*siaA* variant maps within the capsule gene complex (12, 13). This experiment and other restriction fragment analyses indicate a duplicative event for transposition of IS*1301*, since only one additional band was observed after transposition to *siaA*, without other changes in the hybridization pattern. This mechanism has been described for other insertion sequences (14). Several copies of the element from 12 different locations cloned from a genomic library as described in the next section were sequenced, and no sequence diversity was found.

Analysis of the consensus insertion site of IS*1301*. To determine the target site specificity for this element and to search for insertions within known genes, we constructed a genomic library and screened clones for hybridization to the amplified IS sequence. Sequence analysis of 51 positive clones revealed 12 different insertions in addition to the previously described one within *siaA*, the first gene of the capsule biosynthesis pathway encoding a glucose epimerase (Fig. 2). A database search revealed that one insertion site was located upstream of *frpC*, a gene encoding an iron-regulated protein in *N. meningitidis* similar to the RTX cytotoxins (40, 41). The other insertion sites were designated RHA to RHL.

Two consensus core sequences could be found: 5′-ACT AG-3′ was the most favored target (9 of 13 sites) besides the

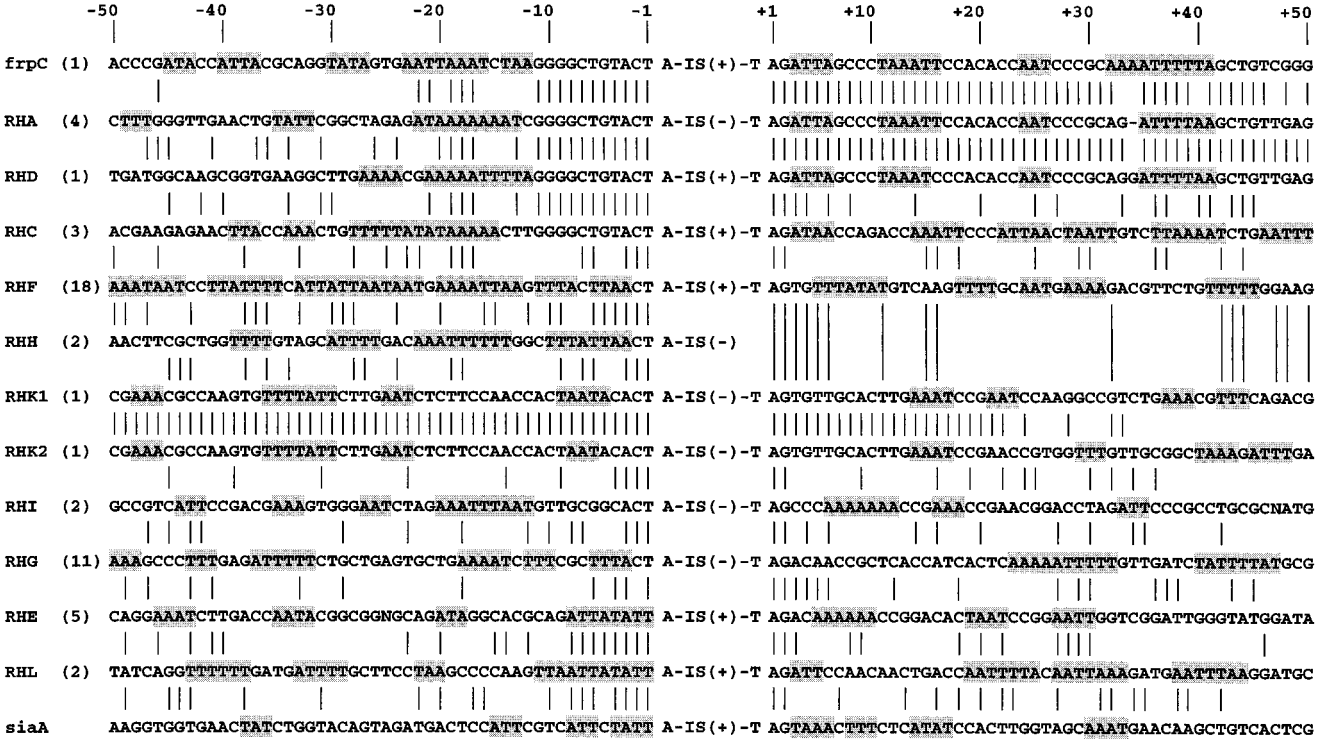

FIG. 2. Insertion sites for IS*1301* in *N. meningitidis* B1940. An alignment of 12 different insertion sites of IS*1301* in *N. meningitidis* B1940 in addition to the temporary insertion site within *siaA* is shown. Homologies relative to the surrounding sequences are indicated. Homologies in sites *frpC*, RHA, RHC, and RHD correspond to the insertion sequence IS*1016*N. AT-rich stretches with three or more bases are shaded. The orientation of the insertion sites was chosen for best possible alignment relative to the known sequences of *siaA* and *frpC*. Insertion of IS*1301* and TA target duplication occur between positions −1 and +1; orientation of IS*1301* within the target site is indicated in parentheses (+ and −, orientation the same as or opposite to that of the published IS sequence, respectively). The numbers of analyzed clones carrying each insertion site are given on the left in parentheses.

5′-ATTAG-3′ insertion site (3 of 13 sites) known within *siaA* (Fig. 2 and 3); for one insertion site (RHH in Fig. 2) the complete target sequence could not be determined, since the clone extended only to the internal *Sau*3A restriction site within IS*1301*. Insertion was always within the central TA dinucleotide and, considering the known sequences of *siaA* and *frpC*, was always accompanied by a TA duplication. All ATT AG target sites might also be extended upstream to the consensus 5′-ATTNTATTAG-3′ sequence (Fig. 2), which can be found only once within the *siaA* gene. Obviously, the distribution of the flanking nucleotides is not random, with a predominant thymidine residue at position −6 and a high probability for this base at positions −4 and +5 from the site of insertion (Fig. 2 and 3). In addition, 15 positions in a region spanning nucleotides +10 to −10 are highly unbalanced in base composition, including a striking underrepresentation of guanine residues in positions −4 to +11.

Comparing the insertion sites in *frpC* and RHA, IS*1301* can be found in both orientations within the palindrome CTAG (Fig. 2), while in all of the nonsymmetrical TTAG sites the element was detected in the same orientation, indicating a role of target symmetry for transposition.

Preferred IS*1301* insertion within another IS. A comparison of the sequences flanking insertion sites RHA, RHC, and RHD with known sequences in a database revealed strong homologies to a 713-bp region upstream of the *frpC* gene from *N. meningitidis* (40), while in one clone the homologies extended to within the gene itself (Fig. 2). This 713-bp region was

G	8	31	46	31	38	46	-	8	38	-	-	-	-	-	100	-	25	-	-	42	-	8	8	-	17	25
A	31	15	15	8	8	46	23	-	31	15	100	-	-	100	-	58	8	33	67	17	33	50	25	25	25	25
T	31	23	23	46	38	8	23	92	23	62	-	23	100	-	-	33	42	58	25	8	8	17	8	58	43	-
C	31	31	15	15	15	-	54	-	8	23	-	77	-	-	-	8	25	8	8	33	58	25	58	17	17	50
Consensus	N	N	N	N	N	**G A**	(C)	**T**	N	(T)	**A**	**C T**	**T**	**A**	**G**	(A)	N	(T)	(A)	N	(C)	(A)	(C)	(T)	N	(C)
Position	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13

FIG. 3. Consensus target sequence for 13 insertion sites of IS*1301*. Nucleotides with the greatest probability for a given position relative to the insertion site of IS*1301* (between positions −1 and +1; see also Fig. 2) are shown. Single nucleotides or pairs with probabilities of >90% at a position (boldface), best nucleotides with a probability of between 50 and 90% (in parentheses), and positions with lower values (N) are indicated. Percentages are rounded and may thus not add up to 100. Dashes indicate nucleotides not present.

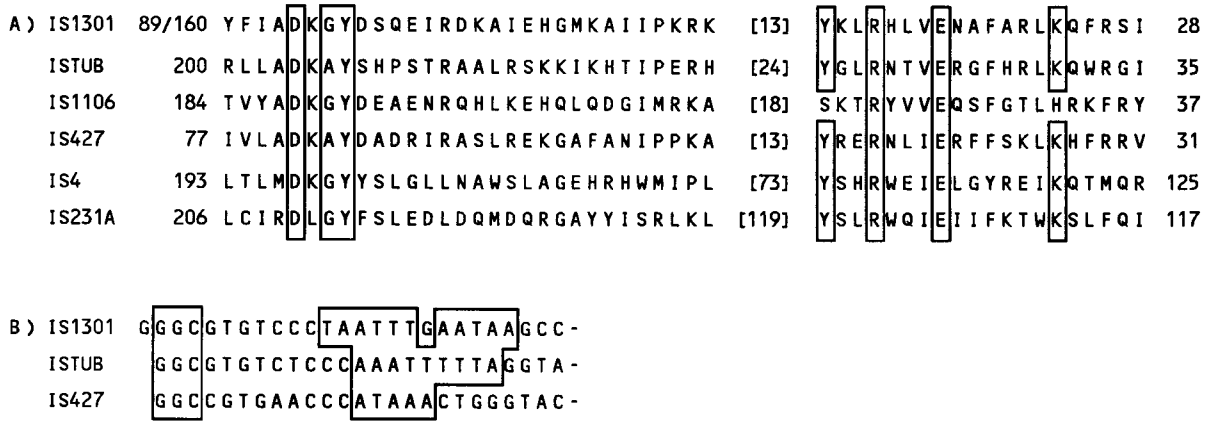

FIG. 4. Comparison of IS*1301* with other elements from the IS*4* family. (A) Alignment of putative transposases. The N3 and C1 regions of selected members of the IS*4*-IS*5* group. The signatures D-(1)-(G/A)-(Y-F) and Y-(2)-R-(3)-E-(6)-K are boxed. The numbers of preceding or following residues of the transposase are indicated on the left and right, for IS*1301*, the two numbers for the preceding residues refer to the single ORF2 and the ORF12 fusion protein, respectively. ISTUB, IS*1106*, and IS*427* belong to the IS*5* subgroup, and IS*4* and IS*231*A are members of the IS*4* subgroup; classification into subgroups is based on the gap lengths between the two signatures (in brackets). (B) Sequence comparison of left IS ends. Members of the IS*5* group share two short patches of sequence conservation (boxed): the external trinucleotide GGC or GAG and an AT-rich motif close to the inner limit of the IR. Only two elements with similar IR are shown for comparison.

described as having 69.6% homology to *Haemophilus influenzae* IS*1016* (8, 23), but no IS assignment has been made for the meningococcal homolog; we refer to this element as IS*1016*N. Interestingly, all insertions of IS*1301* within IS*1016*N were found at the same position in the left IR, although both the left and right IR are identical in nucleotide sequence. Southern hybridizations indicated the presence of at least six copies of this element in *N. meningitidis* B1940 (data not shown) that are highly related, since insertion sites RHA and RHD appear to be almost identical to IS*1016*N (homologies of 94.5 and 94.6% in a 263-bp overlap, respectively, and 91.3% for an alignment of all three sequences), while the homology of site RHC is limited to the IR (Fig. 2).

In addition to the IS*1016*N-like sequences, sites RHK1 and RHK2 are identical over the whole 114-bp sequence determined upstream, and downstream to 22 bp from the insertion, beyond which there are unrelated regions (Fig. 2). Nucleic acid and deduced amino acid sequences of this repeated sequence do not resemble any of the repeats known in *Neisseria* spp. or other known mobile genetic elements (14, 16, 32). Eight base pairs further downstream of the repeated sequence in RHK1, a motif resembling a typical *Neisseria* transcriptional terminator-DNA uptake signal, consisting of the sequence 5′-CGTCTGA AAC-3′ directly followed by its IR, was found (15).

Additional features possibly contributing to IS target specificity. A closer look at the secondary structure around the preferred left IR of the IS*1016*N homologs revealed a putative stem-loop formation (predicted free energy, −7.4 kcal [ca. −31 kJ]/mol; stem size, 5; unpaired bases, 10, as determined by PC/GENE software) presenting the 5′-CTAG-3′ insertion site at the center. In contrast, the right end of the IS and the target site in RHC are not able to form a similar formation because of mismatches in the stem region, since only one side of the stem and the unpaired bases are part of the IR.

A high degree of palindromic symmetry can be observed around eight of the target sites, with 10 or more symmetrical bases between positions −10 and +10, including extended palindromes of 5′-ACACTAGTGT-3′ in sites RHK1 and RHK2 and the 5′-ACTAGT-3′ motif in site RHF (Fig. 2).

It was demonstrated previously that for some transposases, site recognition is in part mediated by homologies of the target site to the ends of the IR (14). Of the 12 insertion sites and the target within *siaA*, only those within the IR of IS*1016*N resemble the ends of IS*1301*, which may additionally contribute to a preferred transposition to those sites. The regions surrounding the target sites are comparatively rich in AT nucleotides, with average AT contents of 59.0% (range, 50 to 76%) and 61.5% (range, 49 to 77%) in the regions spanning bases +100 to −100 and +50 to −50, respectively. In contrast, the average AT content of *Neisseria* spp. is 52%. Of special interest is the high number of long $(A/T)_{n\geq 3}$ tracts in the vicinity of the target sites (Fig. 2), as those tracts are mainly responsible for stable DNA curvature (30). However, the distribution of the described features did not reveal a common pattern in all insertion sites.

IS*1301* is a member of the IS*4* family of elements. Certain features suggest that IS*1301* is related to the IS*4* family of elements, which includes at least 45 elements from very heterogeneous bacterial sources (32). Members of the IS*4* family exhibit only a limited degree of similarity at the nucleotide sequence level, but there are two highly conserved amino acid signatures within the putative transposase: a carboxy-terminal box (C1) with a Y-(2)-R-(3)-E-(6)-K motif and an amino-terminal region (N3) with a D-(1)-(G/A)-(Y/F) consensus core. IS*1301* matches both regions, with a 35-amino-acid spacing between the domains (Fig. 4A). The IS*4* family is further subdivided into the IS*4* and IS*5* subgroups according to the spacing of the N3 and C1 domains and to sequence homology of the IR. We propose placing IS*1301* in the IS*5* subgroup for two reasons: (i) the 35-amino-acid spacer matches the criteria for the IS*5* subgroup (<55 amino acids) (IS*4* subgroup, >80 amino acids), and (ii) the IR of IS*1301* have homologies to ISTUB from *Mycobacterium tuberculosis* and to IS*427* and IS*869*, both from *Agrobacterium tumefaciens*, all belonging to subgroup IS*5* (Fig. 4B) (32).

Distribution of IS*1301* in *N. meningitidis* serogroups. For a limited survey of distribution of IS*1301*, we screened 118 strains of *N. meningitidis* serogroups A, B, C, Y, and W135 from different sources in Europe, three strains of *N. gonorrhoeae*, and 14 strains of apathogenic *Neisseria* spp. for the presence of IS*1301* either by PCR or by colony blotting (Table 1). Interestingly, IS*1301* was found only in meningococci of serogroups B, C, W135, and Y, all of which express a capsule made of polysialic acids (7). Approximately 30% of *N. meningitidis* se-

TABLE 1. Distribution of IS*1301* in *Neisseria* strains

Species and serogroup	No. of strains		Distribution (%)
	Total	Positive for IS*1301*	
N. meningitidis			
A	8	0	0
B	43	12	27.9
C	40	12	30
Y	15	13	86.7
W135	12	4	33.3
N. gonorrhoeae	3	0	0
Neisseria spp.[a]	14	0	0

[a] Including two strains each of *N. elongata*, *N. flava*, *N. lactamica*, *N. mucosa*, *N. perflava*, *N. sicca*, and *N. subflava.*

rogroup B, C, and W135 strains carried the IS, but IS*1301* was found in 86.7% of the group Y strains.

DISCUSSION

In this study, we determined the insertion sites of 12 copies of IS*1301* in *N. meningitidis* B1940 to elucidate the insertion specificity of this element. The consensus core sequence 5′-AYTAG-3′ was determined as the target for transposition. The majority of IS*1301* insertions are located within the least abundant tetranucleotide found in bacterial genomes, CTAG; the frequency in *E. coli* is only 0.02%, compared with 0.4% for a random tetranucleotide (3, 20). Besides including a stop codon, the sequence CTAG is known as a crucial component of binding sites for repressor proteins such as the *metJ* protein and the *trpR* tyrosine repressors (6), and the possibility of DNA kinking around the tetranucleotide at these binding sites has been discussed elsewhere (28). The functional impact of this palindrome is supported by the presence of the DCM (DNA cysteine methylase)-VSP (very short patch) repair mechanism, which leads to its underrepresentation by fixation of point mutations within a CTAG, resulting in overrepresentation of CCAG and CTGG (25).

The transposition of IS*1301* into the *siaA* gene of the capsule biosynthesis pathway of serogroup B meningococci appears to be specific to a single position containing the ATTAG target site (19). However, since there are a number of consensus target sites in *siaA* and the genes of the capsule gene cluster (9) in which insertion of IS*1301* could not be detected so far, the consensus sequence may not be sufficient for target recognition in this location. Extending the consensus sequence by the four conserved positions upstream found in all targets based on the ATTAG core as found in *siaA* results in a sufficient specificity, since this sequence can be found only once in *siaA* at the insertion site. The alignment of target sites was based on the known orientation of the *siaA* and *frpC* genes, but as IS*1301* was found in both directions within the CTAG target of IS*1016*N, we cannot predict the orientations of the other insertions in this palindrome.

Because of the unusually high incidence of factors mediating the secondary structure of DNA flanking IS*1301* insertions, we suggest the need for additional mechanisms for target recognition of the IS*1301*-encoded transposase besides the consensus target sequence. The observed features include extended palindromic symmetry, the stem-loop structure found in IS*1016*N, and long AT tracts, which are known to mediate stable DNA bending (30). However, no distinct pattern indicating DNA secondary structure common to all insertion sites could be identified, as was described to be necessary for the transposition of IS*231*A (17). DNA secondary structure has already been considered to be involved in site recognition for IS*630* (39) and IS*231*A (17) and also for IS*1*, an IS lacking a conserved target sequence (14). Interestingly, IS*231*A preferentially transposes to only one of the IR of another mobile genetic element (Tn*4430*) as well, similar to the IS*1301* insertion in IS*1016*N, and in both cases DNA secondary structures seem to be involved. Nevertheless, since not all of the target sites exhibit all of the features mentioned above except the target consensus 5′-AYTAG-3′, the contribution of each factor to target recognition remains unclear. Because of the limited resolution of restriction assays, we cannot exclude the presence of more than 12 IS*1301* copies in the genome and thus a greater number of target sites not determined by the cloning strategy. However, hybridizations following digestion with 12 different restriction enzymes did not present evidence for a higher copy number (data not shown).

It is interesting that detection of IS*1301* was limited to *N. meningitidis* serogroups expressing a capsule made of polysialic acids. *siaA* is an essential gene of the sialic acid biosynthesis pathway in these serogroups, and this gene is not found in other *N. meningitidis* strains (12). Thus, the coincidence of IS*1301* and *siaA* is notable, since the reversible insertional inactivation of *siaA* is the only observed function of this element so far. The relatively low prevalence of IS*1301* in about 30% of *N. meningitidis* strains of serogroups B, C, and W135 and the presence of completely identical copies in strain B1940 may provide evidence that this element evolved only recently, since minor nucleotide divergences between copies in a single strain are observed for many other IS. It remains unclear why this element is not distributed more widely in *N. meningitidis* strains, although the naturally competent *Neisseria* spp. are known for extensive horizontal gene transfer (11). We also do not know whether the detection of IS*1301* in 86.7% of serogroup Y strains is representative for this group or due to the limited number of strains investigated. Further investigations are necessary to determine whether the target pattern of IS*1301* insertions is similar in serogroups other than serogroup B and if IS*1301* is involved in capsule phase variation of this serogroup as well.

According to amino acid sequence comparisons, we placed our IS in the IS*5* subgroup of the IS*4* family of elements. The only other IS characterized in *N. meningitidis* so far, IS*1106*, has been placed in the same group (22, 32). IS*4* IS are found in very heterogeneous bacterial sources, and thus distribution by horizontal gene transfer has been proposed. This mechanism is widely used in *Neisseria* species (11). The transposases of the IS*4*-IS*5* group of elements are encoded on a single ORF, as is the case for IS*231*A-F, or two overlapping ORFs are fused by a +1 or +2 translational frameshift, as in IS*231*V and IS*231*W, respectively (32). In our first report (19), we proposed a −1 frameshift for fusion of the two ORFs as described for the IS*1* and IS*3* families (4, 10, 35), resulting in a putative transposase of 250 amino acids, but the exact mechanism has not been determined yet. In contrast to other elements, in which the overlap of the two fused ORFs is short (between 16 [IS*711*] and 26 [IS*630*] amino acids) (18, 35), the overlap in IS*1301* is extensive, spanning 81 amino acid residues. How this feature may influence a frameshift is not known, but the presence of previously described slippery codons in the overlapping regions may be indicative of such a fusion (4). It is still possible that both ORFs are transcribed and act separately, since all common characteristics described for transposases, such as the D,D-(35)-E motif found in viral integrases as well as in bacte-

rial insertion sequences (24, 31), and the IS*4*-specific motifs described above are present in ORF2 alone without the need for a fused ORF1. For other elements, a separate transcription was observed, but only the fused ORFs were able to form a functional transposase (4).

The location of one copy 1 kb upstream of the most likely promoter for *frpC*, a gene coding for a protein similar to the RTX cytotoxins (40, 41), and one copy associated with a putative transcriptional terminator allows more questions about additional functions of IS*1301* in gene regulation to be addressed. The activation of genes by transposable elements is well documented, and at least two studies presented evidence that insertional regulation of gene expression can act over greater-than-expected distances spanning more than 700 bp (5, 34).

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